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EVALUATION OF EFFECTIVE BIOCONTROLAGENTS AND ORGANIC AMENDMENTS AGAINST ROOT ROT INCIDENCE OF SESAME THAT INCITED BY *MACROPHOMINA PHASEOLINA* (TASSI) GOID AND ITS RHIZOSPHERE POPULATION

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Abstract

Sesame (*Sesamum indicum* L.) adorned as "Queen of oilseeds" and is affected by a number of diseases, of which the root rot \ is the most serious disease caused by *Macrophomina phaseolina*. In this study, *Trichoderma viride* and *Pseudomonas fluorescens* antagonists and Organic amendments were assessed for their ability to reduce the growth of *M. phaseolina*. Among the antagonists tested by seed treatment + soil application, consortium of *T. viride* (Tv_3) and *P. fluorescens* (Pf_5) recorded the minimum incidence of root rot with **(**20.75**%)** and also reduced the pathogen population to 8.75×10^3 cfu g^{-1} and showed a better rhizosphere competence by recording a population of 23.58, 34.65 cfu g^{-1} of *T. viride* (Tv_3) and *P. fluorescens* (Pf_5) respectively. And also, the treatment with basal application of mahua oil cake @ 500 kg/ha + seed treatment (10 ml/kg) + soil application of consortium (3 lit/ha) (Tv_3+ Pf_5) recorded the least root rot incidence of 18.50%; reduced the pathogen population to (11.02 x $l0^3$) and showed the better competence by recording a rhizosphere population of 27.45 x 10^3, 33.62 x 10^6 cfu g^{-1} soil of *T. viride* and *P. fluorescens*. The results of the present study have proved that application with combination of biocontrol agent's *viz.*, *T. viride* (Tv_3), *P. fluorescens* (Pf_5) plus Mahua oil cake exhibited a general trend towards greater suppression of sesame root rot caused by *M. phaseolina*.

Key words : *Sesamum indicum, Macrophomina phaseolina,* Rhizosphere.

Introduction

Sesame (*Sesamum indicum* L.) is one of the oldest oil seed crop and is under cultivation from ancient times (Weiss, 1971). India contributes the highest sesame acreage of above 17.73 lakh hectare and production 8 lakh tones and productivity of 445kg/hectare (Gupta *et al.*, 2018). The yield of sesame has been affected by several abiotic and biotic factors. Sesame is known to be affected by as many as 80 diseases among which 29 have been reported in India (Vyas et al. 1984). Among these, root rot caused by *Macrophomina phaseolina* (Tassi) Goid is the most serious one affecting the crop, with reports up to 50 per cent incidence resulting in heavy yield losses (Balabaskar *et al.*, 2015). The disease is both seed and soil borne and usually infects the crop under dry and warm conditions. The chemical treatment of diseases are known to exert their inherent ill effects like accumulation of residual toxicity, causing environmental pollution and upsetting the biological imbalance in the soil by over killing the non-targeted microorganisms. It is therefore felt essential to develop an effective, cheap and environmentally safe non-chemical method for the management of root rot diseases. For this use of biological methods seems to be the promising alternative method. Members of the genus *Pseudomonas* and *Trichoderma* have been known for their potential antifungal, plant growth promoting and plant defense inducing activities (Zaidi *et al.*, 2004). Application of single antagonist often result in inconsistent management. Hence the combined application of antagonisms would have potential for more extensive colonization of the rhizosphere, more consistent expression of beneficial traits under a broad range of soil conditions and antagonism to large number of pathogens than overcoming some of the problems that occur with application of individual biocontrol agents (Mayer and Roberts, 2002; Balabaskar, 2006). Besides these organisms, in recent years, organic amendments have been used against plant pathogen successfully. Also the application of organic amendments is one of the successful control methods of soil borne diseases through variety of mechanisms such as providing antimicrobial compounds during decomposition (Mansoor, 2007) reduced the inoculum density of the soil borne plant pathogen through changes in the microbial balance by different mechanism (Klein, 2011). Till today no single method is found to be very effective and economical for the effective management of sesame root rot diseases. Hence, an integrated approach would always ensure the maximum suppression of the disease without any deleterious effect on the ecosystem. Therefore, in this study, antagonistic effect of biocontrol agents and efficacy of various organic amendments were evaluated under *in vitro* against *Macrophomina phaseolina* causing stem and root rot of sesame and result are presented here under.

Materials and Methods

Isolation of the pathogen

The pathogen *M. phaseolina* (Tassi) Goid. was isolated from the diseased roots of sesame plants showing the typical root rot symptoms by tissue segment method (Rangaswami, 1972) on potato dextrose agar (PDA) medium. The axenic cultures of the different isolates of the pathogen were obtained by single hyphal tip method (Rangaswami, 1972) and these were maintained on PDA slants for subsequent experiments.

Seed treatment with antagonist

Seeds of sesame (TMV3) were surface sterilized with two per cent sodium hypochlorite for 30 sec rinsed in sterile dist. water and dried overnight. Ten ml of antagonist inoculum was taken in Petri dish. To this, 100 mg of carboxy methyl cellulose (CMC) was added as an adhesive material. One gram of seeds was soaked in 10 ml of antagonistic suspension for 2 hrs and air dried overnight in a sterile Petri dish.

Soil microflora propagules

The composite soil sample from each pot was collected during both the before sowing and after sowing and it is used for estimation of antagonists and pathogen population using serial dilution technique. The media was prepared and sterilized in autoclave. 1g Soil sample was taken in 9 ml sterilized distilled water in the test tube, stirred well and serial dilution were made up to 10^{-3}.

Efficacy of seed treatment plus soil application with the antagonists on plant growth and root rot incidence of sesame (Pot culture)

Sterilized soil was mixed with the pathogen inoculums @ 5 per cent (w/w) and filled in 30 cm earthen pots. The most effective seed treatment and soil application dosages identified in the earlier experiments alone were used for testing the efficacy of combined delivery system of the antagonists. The antagonists meant for soil application were applied to the sterilized soil in pots and incorporated well. Surface sterilized sesame seeds were treated with antagonists as per the schedule. Surface sterilized sesame seeds sown in pot soil mixed with the inoculums of *M. phaseolina* alone served as control. Seed treatment @ 2 g kg^{-1} plus soil drench @ 0.1% with carbendazim served as comparison. The experiments were conducted with three replications in a randomized block design with five pots per replication and three plants per pot. All the observations *viz.*, plant growth parameters, root rot incidence, population of the antagonist and population of pathogen were recorded.

Treatment details

T_1: *T. viride* (Tv_3) seed treatment (5.0 ml kg^{-1} of seed) + soil application (3.0 ml pot^{-1})

T_2: *P. fluorescens* (Pf_5) seed treatment (10.0 ml kg^{-1} of seed) + soil application (3.0 ml pot^{-1})

T_3: *T. viride* (Tv_3) + *P. fluorescens* (Pf_5) seed treatment (10.0 ml kg^{-1} of seed) + soil application (3.0 ml pot^{-1})

T_4: Carbendazim 50% WP as seed treatment @ 2.0 g kg^{-1} + soil drench @ 0.1%

T_5: Control

Effect of combined application with antagonist and Mahua oil cake on the incidence of root rot of sesame (Pot culture)

Sterilized soil was mixed with the pathogen inoculum @ 5 per cent (w/w) level and filled in 30 cm earthen pots. The most effective seed treatment and soil application dosages identified in the earlier experiments alone were used for testing the efficacy of combined delivery system of the antagonist. The antagonist and oil cake meant for soil application were applied to the pots and incorporated well. Surface sterilized sesame seeds were treated with antagonist as per the schedule. Surface sterilized sesame seeds sown in pots soil mixed with inoculum of *M. phaseolina* alone served as control and soil drenched with Carbendazim (0.1%) was used for comparison. The experiment was conducted with three replications in a randomized block design with three plants per pots were maintained all the observations viz., plant growth parameters, root rot incidence and population of pathogen were recorded as already indicated in the earlier chapter. The best results obtained from the pot culture experiment were selected to study the ISR.

Treatment schedule

T_1: *T. viride* (Tv_3) seed treatment (5.0 ml $kg^{-1}$of seed) + soil application (3.0 ml pot^{-1})

T_2: *P. fluorescens* (Pf_5) seed treatment (10.0 ml $kg^{-1}$of seed) + soil application (3.0 ml pot^{-1})

T_3: *T. viride* (Tv_3) + *P. fluorescens* (Pf_5) seed treatment (10.0 ml kg^{-1} of seed) + soil application (3.0 ml pot^{-1})

T_4: Soil application of Mahua oil cake @ 25 g/pot

T_5: $T_1 + T_4$

T_6: $T_2 + T_4$

T_7: $T_3 + T_4$

T_8: Carbendazim 50% WP as seed treatment @ 2.0 g kg^{-1} + soil drench @ 0.1%

T_9: Control

Results and discussion

Effect of seed treatment and soil application with antagonist on the root rot incidence and rhizosphere population of antagonists and *M. phaseolina* (Pot culture)

The results obtained on the efficacy of combined delivery system of the antagonists *viz.*, seed plus soil treatment are furnished in table 1. Among the antagonists tested by seed treatment + soil application, consortium of *T. viride* (Tv_3) and *P. fluorescens* (Pf_5) recorded the minimum incidence of root rot with **(**20.75**%)** which was on par with the fungicide treatment and also reduced the pathogen population to 8.75×10^3 cfu g^{-1} and showed a better rhizosphere competence by recording a population of 23.58, 34.65 cfu g^{-1} of *T. viride* (Tv_3) and *P. fluorescens* (Pf_5) respectively. Similar to the present study, the combined application of *T. viride* and *P. fluorescens* reduced the population of *M. phaseolina* and root rot incidence and increases the rhizosphere population of antagonists and the biometrics of sesame was reported (Thirunarayanan, 2017). Similarly, Jain *et al*., (2015) investigated application of three microbe consortium in pea that resulted improved growth and yield with reduced disease compared to control and single and double microbe applications following challenge with the pathogen *Sclerotinia sclerotiorum.*

The reduction in the population of the pathogen might be attributed to the activity of the increased rhizosphere population of antagonists and the toxic metabolites produced by the bio agents and organic amendments which might have suppressed the pathogen. The mechanisms by which the antagonists act upon pathogens include antibiotic production, competitive ability, parasitism and lysis (Baker and Cook, 1974; Bull *et al.,* 1991; Raaijmakers *et al.,* 1997).

Effect of combined application of antagonist and mahua oil cake on the root rot incidence and rhizosphere population of antagonists and *M. phaseolina* (Pot culture)

The results on the efficacy of antagonists both individually and in combination with mahua oil cake against root rot incidence were recorded in table 2. The treatment with basal application of mahua oil cake @ 500 kg/ha + seed treatment (10 ml/kg) + soil application of consortium (3 lit/ha) (Tv_3+ Pf_5) recorded the least root rot incidence of 18.50%; reduced the pathogen population to (11.02 x $l0^3$) and showed the better competence by recording a rhizosphere population of 27.45 x 10^3, 33.62 x 10^6 cfu g^{-1} soil of *T. viride* and *P. fluorescens*. Similar to the present study Balabasker, (2006) reported that the application of FYM along with antagonists significantly reduced the pathogen population and root rot incidence and increases the rhizosphere population of antagonists and the biometrics of sesame. Similarly, Sanjeev Kumar *et al.,* (2018) reported that the treatment combination with *S. marcescens,* FYM and micronutrient mixture (T_{12}) showed the best rhizosphere competence and recorded the maximum population of *S. marcescens* with 39.23 x 10^{-6} cfu g^{-1} soil followed by T6 (36.67 x 10^{-6} cfu g^{-1} soil). The same treatment with FYM plus *S. marcescens* (SA + ST) plus micronutrient mixture (SA + ST) significantly reduced the rhizosphere population of *F. oxysporum* f. sp. *cubense* to the minimum with 9.01 from 30.15 x 10^3 cfu g^{-1} soil.

Application of organic amendment to soil is generally a recognized practice for affect disease control by improving the nutrition of the host, thus increasing its resistance, or by reducing the inoculum potential of soil-borne pathogens in the soil and also enhancing better plant growth and yield (Abdel-lateif and Bakr, 2018). Besides, oil cakes might have served as an ideal food base for the growth and multiplication of antagonists and thus led to increased biocontrol activities of the antagonists as reported by Sanjeevkumar and Balabaskar (2010). Organic amendments increased the rhizosphere population of the antagonists

(Ashwani Tapwal *et al.,* 2014). Such increase in the population of the antagonist due to oilcake supplementation might be attributed as the reason for the enhanced disease suppression.

Conclusion

Table 1: Effect of combined application of antagonist and mahua oil cake on the rhizosphere population of antagonists and *M. phaseolina* (Pot culture).

Tr. No	Treatment	Per cent root rot incidence	Per cent reduction	Rhizosphere population (cfu g^{-1} of oven dry soil)		
				Tv (10^3 cfu)	Pf (10^5 cfu)	Mp (10^3cfu)
T_1	*T. viride* ST @ 5 ml kg^{-1} of seed + SA @ 3 ml pot^{-1}	27.50 (31.62)	56.14	29.35	-	17.34
T_2	*P. fluorescens* ST @ 10 ml $kg^{-1}$of seed + SA @ 3 ml pot^{-1}	25.45 (30.29)	59.40	-	37.08	16.94
T_3	Consortium ST @ 10 ml $kg^{-1}$of seed + SA @ 3 ml pot^{-1}	20.36 (26.82)	67.52	25.68	31.75	13.05
T_4	Soil application of Mahua oil cake @ 25 g pot^{-1}	30.60 (33.58)	51.19	4.30	9.18	28.40
T_5	$T_1 + T_4$	22.10 (28.04)	64.75	35.34	-	16.24
T_6	$T_2 + T_4$	21.46 (27.59)	65.77	_	40.20	14.84
T_7	$T_3 + T_4$	18.50 (25.47)	70.49	27.45	33.62	11.02
T_8	Carbendazim 50 % WP as ST @ 2 g kg^{-1} of seed and soil drenched @ 0.1 %	19.00 (25.84)	69.69	-	-	11.83
T_9	Control	62.70	-	-	-	30.26
	S. Ed	0.12	-	0.01	0.01	0.03
	CD (p=0.05)	0.25	-	0.03	0.02	0.08

Data in parentheses indicate angular transformed values.

Table 2: Effect of seed treatment and soil application with antagonist on the root rot incidence and rhizosphere population of antagonists and *M. phaseolina* (Pot culture).

Tr. No	Treatment	Per cent root rot incidence	Per cent reduction	Rhizosphere population (cfu g^{-1} of oven dry soil)		
				Tv (10^3 cfu)	Pf (10^5 cfu)	Mp (10^3cfu)
T_1	*T. viride* ST@ 5 ml kg^{-1} of seed + SA @ 3 ml pot^{-1}	25.30 (30.19)	63.34	24.50	-	11.85
T_2	*P. fluorescens* ST @ 10 ml kg^{-1} of seed + SA @ 3 ml pot^{-1}	23.16 (28.76)	66.44	-	36.75	10.09
T_3	Consortium ST@ 10 ml kg^{-1} of seed + SA @ 3 ml pot^{-1}	20.75 (27.09)	69.93	23.58	34.65	8.75
T_4	Carbendazim ST @ 2 g kg^{-1} of seed	21.10 (27.34)	69.42	-	-	7.90
T_5	Control	69.02 (56.17)	-	-	-	27.15
	S. Ed	0.24	0.11	0.01	0.02	0.01
	CD (p=0.05)	0.65	0.26	0.03	0.05	0.03

Data in parentheses indicate angular transformed values.

In most research to date, biocontrol agents are applied singly to combat a pathogen. But the results of the present study have proved that application with combination of biocontrol agent's *viz.*, *T. viride* (Tv_3), *P. fluorescens* (Pf_5) plus Mahua oil cake exhibited a general trend towards greater suppression of sesame root rot caused by *M. phaseolina*. Such enhanced suppression exerted by combination of biocontrol agents and organic amendments may be due to the combined action of different mechanisms and better performance of antagonists in varied microclimates and seasons.

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